

CLAIMS

1. A single domain ligand consisting of at least part of the variable domain of one chain of a molecule from the immunoglobulin (Ig) superfamily.
2. The ligand of claim 1, which consists of the variable domain of an Ig heavy chain.
3. The ligand of claim 1, which consists of the variable domain of an Ig chain with one or more point mutations from the natural sequence.
4. A receptor comprising a ligand of any one of claims 1 to 3 linked to one or more of an effector molecule, a prosthetic group, a label, a solid support or one or more other ligands having the same or different specificity.
5. The receptor of claim 4, comprising at least two ligands.
6. The receptor of claim 5, wherein the first ligand binds to a first epitope of an antigen and the second ligand binds to a second epitope.
7. The receptor of claim 6, which includes an effector molecule or label.
8. The receptor of ~~any one of claims 5 to 7~~ which comprises a ligand and another protein molecule, produced by recombinant DNA technology as a fusion product.
9. The receptor of claim 8, wherein a linker peptide sequence is placed between the ligand and the other protein molecule.
10. A method of cloning a sequence (the target sequence) which encodes at least part of the variable domain of an Ig

superfamily molecule, which method comprises:

- (a) providing a sample of double stranded (ds) nucleic acid which contains the target sequence;
- (b) denaturing the sample so as to separate the two
5 strands;
- (c) annealing to the sample a forward and a back oligonucleotide primer, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of the target sequence, the back primer being specific for
10 a sequence at or adjacent the 3' end of the antisense strand of the target sequence, under conditions which allow the primers to hybridise to the nucleic acid at or adjacent the target sequence;
- (d) treating the annealed sample with a DNA polymerase
15 enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place; and
- (e) denaturing the sample under conditions such that the extended primers become separated from the target sequence.
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11. The method of claim 10, further including the step (f) of repeating steps (c) to (e) on the denatured mixture a plurality of times.
- 25 12. The method of claim 10 or ~~claim 11~~, which is used to clone a complete variable domain from an Ig heavy chain.
13. The method of claim 10 or ~~claim 11~~ which is used to produce a DNA sequence encoding a ligand according to any
30 one of claims 1 to 3.
14. The method of ~~any one of claims 10 to 13~~, wherein the forward and back primers are provided as single oligonucleotides.
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15. The method of ~~any one of claims 10 to 13~~, wherein the forward and back primers are each supplied as a mixture of closely related oligonucleotides.

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16. The method of claim 14 or claim 15, wherein the primers which are used are species specific general primers.

5 17. The method of ~~any one of claims 10 to 16~~, wherein the ds nucleic acid sequence is genomic DNA.

18. The method of ~~any one of claims 10 to 17~~, wherein the ds nucleic acid is derived from a human.

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19. The method of ~~any one of claims 10 to 18~~, wherein the ds nucleic acid is derived from peripheral blood lymphocytes.

15 20. The method of ~~any one of claims 10 to 18~~, wherein each primer includes a sequence encoding a restriction enzyme recognition site.

21. The method of claim 20, wherein the restriction enzyme
20 recognition site is located in the sequence which is annealed to the ds nucleic acid.

22. The method of ~~any one of claims 10 to 21~~, wherein the product ds cDNA is inserted into an expression vector and
25 expressed alone.

23. The method of ~~any one of claims 10 to 22~~, wherein the product ds cDNA is expressed in combination with a complementary variable domain.

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24. The method of ~~any one of claims 10 to 23~~, wherein the cloned ds cDNA is inserted into an expression vector already containing sequences encoding one or more constant domains to allow the vector to express Ig-type chains.

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25. The method of ~~any one of claims 10 to 24~~, wherein the cloned ds cDNA is inserted into an expression vector so that it can be expressed as a fusion protein.

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26. The method of claim 10, wherein one or both of the primers comprises a mixture of oligonucleotides of hypervariable sequence, whereby a mixture of variable domain encoding sequences is produced.

27. A method of cloning a sequence (the target sequence) which encodes at least part of the variable domain of an Ig superfamily molecule, which method comprises:

- 10 (a) providing a sample of double stranded (ds) nucleic acid which contains the target sequence;
- (b) denaturing the sample so as to separate the two strands;
- (c) annealing to the sample a forward and a back oligonucleotide primer, the forward primer being specific for a sequence at or adjacent the 3' end of the sense strand of the target sequence, the back primer being specific for a sequence at or adjacent the 3' end of the antisense strand of the target sequence, under conditions which allow the primers to hybridise to the nucleic acid at or adjacent the target sequence;
- 15 (d) treating the annealed sample with a DNA polymerase enzyme in the presence of deoxynucleoside triphosphates under conditions which cause primer extension to take place;
- 20 (g) treating the sample of ds cDNA with traces of DNase in the presence of DNA polymerase I to allow nick translation of the DNA; and
- 25 (h) cloning the ds cDNA into a vector.

30 28. The method of claim 27, which further includes the steps of:

- (i) digesting the DNA of recombinant plasmids to release DNA fragments containing genes encoding variable domains; and
- 35 (j) treating the fragments in a further set of steps (c) to (h).

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29. The method of either claim 27 or ~~claim 28~~, wherein the fragments are separated from the vector and from other fragments of the incorrect size by gel electrophoresis.

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5 30. The method of ~~any one of claims 27 to 29~~, wherein the product ds cDNA is cloned directly into an expression vector.

III (w D)
31. A species specific general oligonucleotide primer or
10 mixture of such primers useful for cloning at least part of a variable domain encoding sequence from an animal of that species.

15 32. A primer or mixture of primers according to claim 27, wherein each primer includes a restriction enzyme recognition site within the sequence which anneals to the coding part of the variable domain encoding sequence.

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